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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/852,000	05/10/2001	Takashi Osumi	046124-5005-02-US	4124

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EXAMINER	
SLOBODYANSKY, ELIZABETH	
ART UNIT	PAPER NUMBER
1652	

DATE MAILED: 10/22/2002 11

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/852,000	OSUMI ET AL.	
	Examiner	Art Unit	
	Elizabeth Slobodyansky	1652	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *10 July 2002* .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *8-20* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *8-20* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *10 July 2002* is/are: a)☐ accepted or b)☒ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☒ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s) *7* .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

DETAILED ACTION

The amendment filed July 10, 2002 amending the specification to insert reference to SEQ ID NO: 15 and amending claims 8, 10, 11, 13, 15 and 16 has been entered.

The examiner notes that directions for entry of SEQ ID NO:15 was defective because the entire paragraph containing the sequence should have been provided in clean form. Further, the sequence of the current SEQ ID NO:15 has not been previously designated as SEQ ID NO:1 as indicated by the amendment.

Claims 8-20 are pending.

Drawings

The new drawings filed July 10, 2002 have been objected by Draftsman, please refer to the attached form PTO-948 for details.

Specification

This application contains sequence disclosures that are encompassed by the definitions for nucleotide and/or amino acid sequences set forth in 37 CFR 1.821(a)(1) and (a)(2). However, this application fails to comply with the requirements of 37 CFR 1.821 through 1.825. 37 CFR 1.821(d) requires the use of assigned sequence

identifier in all instances where the description or claims of a patent application discuss sequences.

The following are examples of incompliance where the sequence containing more than ten nucleotides or four amino acids is given without a sequence identifier: sequences shown on pages 15-16, 21.

Correction is required.

The disclosure is objected to because of the following: It appears that the basic plasmid used for the production of BFP variants contains mutations S65T, H231L and a valine inserted between Met1 and Ser2 (pages 14, line 17, through page 17, line 21). However, the specification on page 23, line 13, describing BFP does not mention the above three mutations.

Clarification is required.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 8, 10, 11, 13, 15, 16, with dependent claims 17-20, are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in

the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Claims 8, 10, 11, 13, 15 and 16 are directed to a DNA encoding a fluorescent protein comprising the amino acid sequence set forth in SEQ ID NO:1 with at least mutations of Phe64Leu, Val163Ala and Ser175Gly; Phe64Leu, Tyr66His and Tyr145Phe; Phe64Leu, Tyr66His, Tyr145Phe and Leu236Arg; Phe64Leu, Tyr66His, Tyr145Phe, Val163Ala, Ser175Gly and Leu236Arg; Val163Ala and Ser175Gly; and Tyr66His, Tyr145Phe, Val163Ala and Ser175Gly , respectively. Claims 17-20 are dependent claims and are drawn to methods of use thereof.

The specific recited mutations constitute no more than 2.9% of the entire SEQ ID NO:1 that is 238 amino acids long. The use of "at least mutations" renders the claims to encompass DNAs encoding a fluorescent protein having any structure and any fluorescent characteristics as long as its structure comprises the above mutations. Therefore, the claims are drawn to a genus of DNAs encoding fluorescent proteins described by insufficient limitations on either structure or function. The specification discloses no identifying characteristics which would allow to recognize a structure as exhibiting any fluorescence.

The specification teaches the structure of only a single representative species of such DNAs. Moreover, the specification fails to describe any other representative

species by any identifying characteristics or properties other than the functionality of encoding a fluorescent protein.

Therefore, based on the instant disclosure, it is unpredictable either a protein is a fluorescent protein. Thus, a DNA encoding a fluorescent protein comprising the amino acid sequence set forth in SEQ ID NO:1 with at least mutations of Phe64Leu, Val163Ala and Ser175Gly; Phe64Leu, Tyr66His and Tyr145Phe; Phe64Leu, Tyr66His, Tyr145Phe and Leu236Arg; Phe64Leu, Tyr66His, Tyr145Phe, Val163Ala, Ser175Gly and Leu236Arg; Val163Ala and Ser175Gly; or Tyr66His, Tyr145Phe, Val163Ala and Ser175Gly, lacks sufficient written description needed to practice the invention of claims 8, 10, 11, 13 and 15-20.

Claims 8, 10, 11, 13 and 15-20 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a DNA encoding a fluorescent protein having the amino acid sequence of SEQ ID NO:1 consisting of mutations Phe64Leu, Val163Ala and Ser175Gly; Phe64Leu, Tyr66His and Tyr145Phe; Phe64Leu, Tyr66His, Tyr145Phe and Leu236Arg; Phe64Leu, Tyr66His, Tyr145Phe, Val163Ala, Ser175Gly and Leu236Arg; Val163Ala and Ser175Gly; or Tyr66His, Tyr145Phe, Val163Ala and Ser175Gly, does not reasonably provide enablement for a DNA encoding a fluorescent protein having the amino acid sequence of SEQ ID NO:1 with at least mutations Phe64Leu, Val163Ala and Ser175Gly; Phe64Leu, Tyr66His and

Tyr145Phe; Phe64Leu, Tyr66His, Tyr145Phe and Leu236Arg; Phe64Leu, Tyr66His, Tyr145Phe, Val163Ala, Ser175Gly and Leu236Arg; Val163Ala and Ser175Gly; and Tyr66His, Tyr145Phe, Val163Ala and Ser175Gly. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with these claims.

Factors to be in In re Wands 858 F.2d 731, 8 USPQ2nd 1400 (Fed. Cir. 1988). They include (1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7)considered in determining whether undue experimentation is required, are summarized the predictability or unpredictability of the art, and (8) the breadth of the claims.

Claims 8, 10, 11, 13 and 15-20 are directed to a DNA encoding a fluorescent protein comprising the amino acid sequence set forth in SEQ ID NO:1 with at least mutations Phe64Leu, Val163Ala and Ser175Gly; Phe64Leu, Tyr66His and Tyr145Phe; Phe64Leu, Tyr66His, Tyr145Phe and Leu236Arg; Phe64Leu, Tyr66His, Tyr145Phe, Val163Ala, Ser175Gly and Leu236Arg; Val163Ala and Ser175Gly; or Tyr66His, Tyr145Phe, Val163Ala and Ser175Gly. This amounts to a DNA encoding any

fluorescent protein having any structure and any fluorescent characteristics as long as its structure comprises the above mutations.

The specification does not support the broad scope of the claims which encompass all modifications and fragments of any sequence that comprises mutations Phe64Leu, Val163Ala and Ser175Gly; Phe64Leu, Tyr66His and Tyr145Phe; Phe64Leu, Tyr66His, Tyr145Phe and Leu236Arg; Phe64Leu, Tyr66His, Tyr145Phe, Val163Ala, Ser175Gly and Leu236Arg; Val163Ala and Ser175Gly; and Tyr66His, Tyr145Phe, Val163Ala and Ser175Gly because the specification does **not** establish: (A) regions of the protein structure which may be modified without effecting the specific requisite activity of the polypeptide of the instant invention; (B) the general tolerance of said polypeptide to modification and extent of such tolerance; (C) a rational and predictable scheme for modifying any amino acid residues with an expectation of obtaining the desired biological function; and (D) the specification provides insufficient guidance as to which of the essentially infinite possible choices is likely to be successful.

Despite knowledge in the art to produce mutations in proteins, the specification fails to provide guidance as to where, and what type of (i.e., what amino acid to substitute into, add to or delete from the known sequence), changes in amino acid residues will result in a desired biological activity. The amino acid sequence of a protein determines its structural and functional properties, and predictability of what

mutations can be tolerated in a protein's sequence and result in a certain activity is extremely complex, and well outside the realm of routine experimentation, because accurate predictions of a protein's function from mere sequence data are limited.

Furthermore, while recombinant and mutagenesis techniques are known, it is not routine in the art to screen large numbers of mutated proteins where the expectation of obtaining similar activity is unpredictable based on the instant disclosure.

Therefore, one of ordinary skill in the art would require guidance, in order to make a DNA encoding a fluorescent protein having any amino acid sequence with at least mutations Phe64Leu, Val163Ala and Ser175Gly; Phe64Leu, Tyr66His and Tyr145Phe; Phe64Leu, Tyr66His, Tyr145Phe and Leu236Arg; Phe64Leu, Tyr66His, Tyr145Phe, Val163Ala, Ser175Gly and Leu236Arg; Val163Ala and Ser175Gly; or Tyr66His, Tyr145Phe, Val163Ala and Ser175Gly in a manner reasonably correlated with the scope of the claims. Without such guidance, the experimentation left to those skilled in the art is undue.

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 9, 12 and 14, with dependent claims 17-20, are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 9, 12 and 14 recite SEQ ID No.1 "with" the specific number of mutations. It is unclear whether the claims are meant to encompass only the recited mutations or may include other mutations as well. In other words it is unclear either open or closed language is implies.

For the purposes of examination, the examiner construed the claim as reciting closed language, i.e. SEQ ID NO:1 with only three, four, etc. mutations.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless --
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.
>
> (e) the invention was described in a patent granted on an application for patent by another filed in the United States before the invention thereof by the applicant for patent, or on an international application by another who has fulfilled the requirements of paragraphs (1), (2), and (4) of section 371© of this title before the invention thereof by the applicant for patent.

Claim 15 is rejected under 35 U.S.C. 102(b) as being anticipated by Siemering et al.

Siemering et al. (form PTO-1449 filed May 10, 2001, reference 12) discloses a DNA encoding a GFP mutant (GFPA) comprising double mutation Val163Ala/Ser175Gly (page 1654, 2nd column). They teach that said mutant has about 4 fold higher fluorescence at 37°C than at 30°C (page 1655, figure 2).

Claim 10 is rejected under 35 U.S.C. 102(e) as being anticipated by Tsien et al.

Tsien et al. (US Patent 6,197,928, form PTO-1449 filed July 10, 2002) teach a DNA encoding the GFP mutant comprising mutations F64L/Y66H/Y145F (column 23, lines 21-35).

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 8 and 9, with dependent claims 17 and 18, are rejected under 35 U.S.C. 103(a) as being unpatentable over Siemering et al. in view of Thastrup et al.

The teachings of Siemering et al. are outlined above.

Thastrup et al. (WO 97/11094, form PTO-1449 filed May 10, 2001, reference 6) teach mutant GFP proteins comprising mutation F64L such as F64L-GFP, F64L-Y66H-GFP and F64L-S65T-GFP that have a cellular fluorescence far exceeding the cellular fluorescence of the parent proteins (page 3, lines 12-15). They teach that said proteins have a higher fluorescence at 37°C than at 22°C (Figures).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to combine mutations Val163Ala/Ser175Gly taught by Siemering et

al. with F64L mutation taught by taught by Thastrup et al. One skilled in the art would have been motivated to combine these mutations on order to make a mutant having an improved fluorescence at higher temperatures. One could have a reasonable expectations of success of at least accumulative effect because both mutants retain their respective properties when combined with other mutations.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to use a cell transfected with a DNA encoding the fluorescent mutant for visually analyzing gene expression or protein localization according to the intended use of such mutants as taught by Siemering et al. and Thastrup et al.

Because the V163A/S175G GFP mutant alone has about 4 fold higher fluorescence at 37°C than at 30°C (Siemering et al., *supra*), the fluorescence exhibited by the 105 mutant (Phe64Leu/Val163Ala/Ser175Gly) that is about 3.5 times higher fluorescence at 37°C than at 30°C is not deemed as an unexpected result (page 31, Table 5).

Claim 10, with dependent claims 19 and 20, is rejected under 35 U.S.C. 103(a) as being unpatentable over Tsien et al. in view of Thastrup et al.

The teachings of Thastrup et al. are outlined above.

Tsien et al. (US Patent 6,197,928, form PTO-1449 filed July 10, 2002) teach a DNA encoding the "blue" fluorescent GFP mutant P4-3 comprising mutations

Y66H/Y145F (column 10, Table 1). They teach that it has dim fluorescence at 37°C (column 23, lines 15-21).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to combine mutations P4-3 mutations taught by Tsien et al. with F64L mutation taught by Thastrup et al. One skilled in the art would be motivated to combine these mutations on order to make a blue fluorescent protein having an improved fluorescence at higher temperatures. One could have a reasonable expectations of success because the addition of F64L to the "original" BFP (Y66H-GFP) resulted in improved fluorescence as taught by Thastrup, *supra*.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to use a cell transfected with a DNA encoding the fluorescent mutant for visually analyzing gene expression or protein localization according to the intended use of such mutants as taught by Tsien et al. and Thastrup et al.

Claim 16, with dependent claims 19 and 20, is rejected under 35 U.S.C. 103(a) as being unpatentable over Tsien et al. in view of Siemering et al.

The teachings of Tsien et al. and Siemering et al. are outlined above.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to combine P4-3 mutations taught by Tsien et al. with Val163Ala/Ser175Gly taught by Siemering et al. which are responsible for

thermostability. One skilled in the art would be motivated to combine these mutations on order to make a BFP with improved fluorescence at higher temperatures. One could have a reasonable expectations of success of an accumulative effect because both mutants, Y66H/Y145F taught by Tsien et al. and Val163Ala/Ser175Gly taught by Siemering et al., retain their respective properties when combined with other mutations.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to use a cell transfected with a DNA encoding the fluorescent mutant for visually analyzing gene expression or protein localization according to the intended use of such mutants as taught by Tsien et al. and Siemering et al.

Allowable Subject Matter

Claims 12 and 14 would be allowable if rewritten to overcome the rejection(s) under 35 U.S.C. 112, second paragraph, set forth in this Office action and to include all of the limitations of the base claim and any intervening claims.

Response to Arguments

Applicant's arguments filed July 10, 2002 have been fully considered but they are not persuasive.

With regards to the written description rejection Applicants argue that "this [residues 65-66-67 of SEQ ID NO:1] grouping of amino acids forms identifiable

structure disclosed in the application as contributing to fluorescence (page 5, 2nd paragraph). This is not persuasive because while the discussed triad is contributing to fluorescence, it is not fluorescent itself. Therefore, the correlation between the structure and fluorescence common to all members of the genus is lacking from the description. Furthermore, the triad essential to fluorescence is known in the art and by itself does not distinguish the claimed genus from the prior art.

Applicants further argue that they disclosed "a representative number of species so as to justify the claimed genus" (page 6, 2nd paragraph). Applicants exemplify their position by discussing mutants 202 and 205 (Table 4) (page 6, last paragraph through page 7, 1st paragraph). The disclosed mutants (either 202 and 205 or others) do not support sufficient written description of the genus because the mutations represent a minuscule percent of the structure that by itself is insufficient to impart fluorescence. There is no description of the rest of the protein structure because allowed mutations in SEQ ID NO:1 are not limited to the mutations present in the disclosed mutants.

With regard to the enablement rejection, Applicants argue that " the isolation of more than one protein within the claimed genus [is enabled] using the methods disclosed in the specification. Indeed, the Application discloses at pages 18-20 a method for randomly introducing mutations into a GFP sequence using Mutagenic PCR, wherein random mutants are screened for increased fluorescence in *E. coli* following UV irradiation" (page 8, 2nd paragraph). Applicants continue "in contrast to

what alleged in the Office Action dated January 10, 2002, the disclosure does provide the expectation that one may successfully obtain other mutants demonstrating fluorescence that contain further mutations in addition to those disclosed in the claims" (page 8, last paragraph, emphasis added). This is not persuasive because Mutagenic PCR and other techniques are known in the art, they are not novel to the instant invention. Obviously, Applicants have already collected all mutants with the requisite properties obtained by the described method. This does not allow to predict what are other "further" mutations that are permissible. This is because the structure is not limited to SEQ ID NO:1 except for the recited mutations. He claims are not drawn to a mutant that comprises the mutations of the specific mutant and has a sequence that is 95% identical to SEQ ID NO:1, for example. Such genus would be enabled.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Elizabeth Slobodyansky whose telephone number is (703) 306-3222. The examiner can normally be reached Monday through Friday from 9:30 AM to 6:00 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Dr. Ponnathapura Achutamurthy, can be reached at (703) 308-3804. The FAX phone number for Technology Center 1600 is (703) 308-4242.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the Center receptionist whose telephone number is (703) 308-0196.

E. Slobodyansky

Elizabeth Slobodyansky, PhD
Primary Examiner
October 16, 2002